Compelling Evidence of Fossils and Microbialites on Ancient Mars

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Edited by

Vincenzo Rizzo and Giorgio Bianciardi

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I dedicate this book to my sweet wife, Graziella.
She brought patience and acceptance into the time stolen from her when I put all my focus into this work.
She brought understanding into the hard times that this work entailed for me.
She deserves my gratitude more than anyone and everyone, as she filled my bizarre life with sweetness and love.

The color of the sea
on the horizon's line,
where gently lie down
the last rays of the sun,

Is not the dark deep blue of the ocean,
Nor the light blue of the sky.
It's so much more:

It's the pale color of your hair,
where I softly lay
my tired sight

It's the color of the infinite
where we fly:
The birds in the sky,
The call of the seagull,
It is our love,

Forever

Vincenzo Rizzo

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G. Bianciardi would particularly like to thank Gilbert Victor Levin (Baltimore, 23 April 1924 – Bethesda, 26 July 2021), Principal Investigator and inventor of the Labeled Release Test on board the Vikings. In my opinion, but also in the opinion of many other astrobiologists, he is the one who, one day, will be recognized as the one who first discovered life on Mars. It was an honor to have worked with him.

PREFACE

VINCENZO RIZZO

The search for extraterrestrial life is one of the most important endeavors of modern science, and Mars is the best candidate for fulfilling it, both in terms of the intrinsic probability that life could have once existed there, and in terms of the availability of information. The NASA rovers Spirit, Opportunity, and more recently Curiosity and Perseverance travelled, taking on the task of geologists, and analyzing former lakes and sea deposits where life may have existed. They have provided many thousands of images and interesting clues as to the possibility of past Martian life.

In Chapter 2, which makes up the central part of this volume, we present a large selection of sedimentary structures recorded by the NASA rovers (before March 2023, the starting date of this book's preparation), through their progressive, reasoned and weighed evaluation, in order to show their congruence with the world of microbialites. Even more pioneering, we aim to show their congruity with some recurrent millimeter shapes, having complex and inexplicable structure, and traits of terrestrial forms of life which resemble microfossils.

The initial suggestion for the title of this book was "Evidence of past life on Mars: microbialites and microfossils". A title that was, and remains, in our opinion, most in line with the content of the book. The difference to the current title is not significant, but merely clarifies the limitations of the terminology. The current version responds to the suggestions of an eminent geologist and NASA collaborator, who was consulted for his input. Although appreciative of the remarkable work done, and the scientific interest of the analyzed structures, he wanted to make clear that such interesting structures cannot be considered proof of life, *as great caution is needed on this topic*, due to the social and religious implications.

Consequently, the question emerges of, What the difference is between the two titles, and What does "evidence" mean? Evidence is the magic link which connects the mind to an object and its context through its vision. Furthermore, it represents the subjective reply to the question: "What is it?" and what it is for us, it may not be for others. To better emphasize the subjectivity of the term “evidence” we added the term “compelling” to the type of evidence.

Despite its subjectivity, ‘evidence’ can assume a very different relevance (and scientific consensus) when the evaluation process is supported by multiple convergent elements, and when the latter lead to only one possible conclusion. In this case the evidence becomes “compelling evidence” (as suggested by several authors, as shown elsewhere in the volume) and the term ‘putative’ indicates a useful proposition, valid for further characterization and in-depth investigations.

At the center of this evaluation there are sciences such as “Sedimentology and Paleontology” which, in our terrestrial environment, are much more varied and dynamic than the Martian one. These sciences give us a broad palette of methods of approach and environment-related products that can be employed to exclude mimic cases and to support the evidence.

The hardest thing to see is what is in front of your eyes.
—Johann Wolfgang von Goethe.

CHAPTER 1

MARS:
THE SEARCH FOR LIFE

GIORGIO BIANCIARDI

State of the art

This chapter aims to present the state of the art involved in the search for life on the Red Planet, underlining the points of greatest interest that have emerged in this research over the last 50 years, since space missions made this study possible. Mapping out the state of the art involved in this endeavor takes us along a path which is far from linear, and which we can begin with the images taken by the first probe to enter the Martian orbit (Mariner 9, 1972). It managed to take images with resolutions high enough to obtain results that detected characteristics of a planet which, in its past, could have hosted forms of life. In fact, in the geological past of Mars, we now know that there existed a period lasting more than a billion years in which the environment was strongly pro-life.

The four ages of Mars

It is widely recognised that on ancient Mars, billions of years ago, liquid water was widespread, volcanic activity and heat flow were present, as well as a global magnetic field that protected against hostile radiation, a thicker atmosphere and temperatures possibly approaching that of the Earth.

To explain how we arrived at the present conditions (Fig. 1-1) we need to delineate the four ages of Mars as they are currently described (Carr, and Head 2010, 185–203):

1) Pre-Noachian (4.5 - 4.1 billion years ago)

A serious hypothesis describes Mars at that time as having a dense atmosphere, a global ocean, and a mean temperature of > 0°C: the first window for the possible emergence of life on Mars around 4.4 to 4.3 billion years ago (Chambers 2014, 479-480).

Fig. 1-1: Mars, Mojave crater. Credit: ESA, Mars Express.

2) Noachian (4.1 – 3.7 billion years ago)

A period of heavy bombardment, with numerous asteroid and comet impacts (as was the case on Earth at the time). Large-scale volcanic activity was taking place, pouring ash and gases into the atmosphere; thus the planet warmed. Precipitation rained down. Without any doubt the conditions remained favorable for life, as proved by the presence in the ALH84001 Martian meteorite of carbonates precipitated in a fluid (water) of 18°C about 4 billion years ago (Halevy, Fischer, and Eiler 2011, 16895–16899).

3) Hesperian (3.7 - 2.9 billion years ago)

Global geological activity was slowing down, although there was still considerable volcanism. Huge amounts of water and sulphur dioxide were emitted by erupting volcanoes, which then got precipitated onto the surface. The climate became colder. Much of the water became locked up as permafrost or subsurface ice, but was ready to erupt onto the surface when heated by impacts, causing catastrophic floods. These were short-lived flash floods that unleashed torrents equivalent to thousands of Mississippi Rivers (Warner, Gupta, Lin, Kim *et al*. 2010, 1-29).

4) Amazonian (2.9 billion years ago - present)

The planet's surface turned dry and arid, punctuated only by occasional, short-lived returns to warmer, wetter conditions. The rocks slowly altered due to weathering. The atmosphere became so thin that water would instantly vaporize on the surface. The current appearance of Mars came into being.

However, the climate and the stability of water at the surface varied over thousands to millions of years as the axial tilt of the planet underwent cyclical changes. At present, Mars is experiencing an Ice Age, but every 50,000 years the atmosphere becomes denser, the temperature increases, and snow (water!) falls all over the planet. In sum, an environment is formed which becomes once again favorable to life.

Vikings; the search for life on Mars starts

The first space missions to Mars (Mariner 4, 6, 7 missions, 1964-1969, NASA) revealed a terrible aspect (for an astrobiologist!) of present-day Mars. Polar caps made of solid carbon dioxide, an atmospheric pressure inferior to 7 mmHg- a condition in which it is impossible for liquid water to be present- a surface made up only of craters: a planet that appeared more similar to the Moon than to the Earth. In effect, those space missions were made by a quick fly-by past the planet with very low-resolution images. A few years later, the prospect of Mars changed into a more interesting one.

Mariner 9 (NASA) reached Mars (1972) and for the first time entered low orbit and took high-resolution photos. Mars was revealed as hosting huge volcanoes, active until recently, and rivers, now dry but which once flowed for thousands of kilometers (Fig. 1-2).

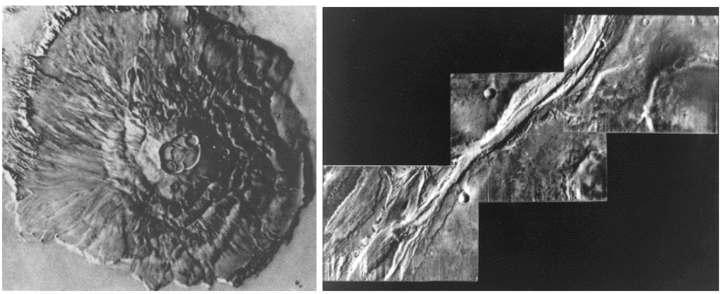

Fig. 1-2: Mariner 9 revealed the presence of huge volcanoes (left) and the imprint of rivers (right) thousands of kilometers long. Credit: NASA.

The number of pro-life data were sufficient to make NASA decide to send real biological laboratories to Mars: the search for life on Mars had begun. Two spacecraft were launched from Cape Canaveral, Florida: Viking 1 on

August 20, 1975, and Viking 2 on September 9, 1975. The landers were heavily sterilized before launch to prevent contamination of Mars with organisms from Earth. The spacecrafts spent nearly one year cruising to Mars.

Viking 1 landed on Mars on July 20, 1976, on the western slope of Chryse Planitia (the Plains of Gold) at 22.3 degrees north latitude, 48.0 degrees longitude. The Viking 2 landing took place on September 3, 1976, at Utopia Planitia, at 47.7 degrees north latitude and 48.0 degrees longitude, 3000 km from where the former, principal mission was to perform biological analyses of the Martian regolith. Three biological tests were performed: Gas Exchange, Pyrolytic Release, and Labeled Release (for a review, see: Chambers 1999). The first two tests gave very ambiguous results, but the third, the Labeled Release test, gave rise to a series of as yet unresolved conjectures on the possibility that the latter had found evidence of Martian microbial life.

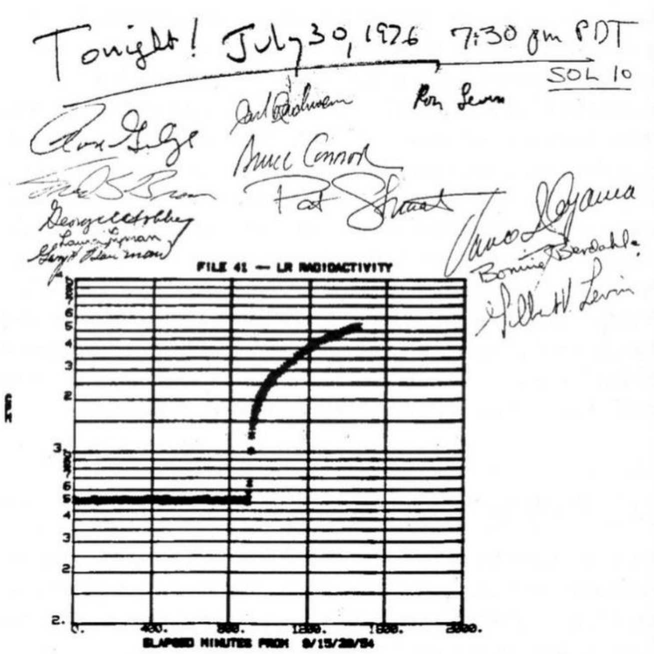

Fig. 1-3: Gilbert V. Levin, Principal Investigator of the Labeled Release test, and his team (and Levin's young son, Ron) sign their success: "Tonight!" (an allusion to the musical "West Side Story"), "…we have discovered life on Mars." Credit: personal courtesy of Gilbert V. Levin.

The Labeled Release test (LR) was a very simple analysis to test for the presence of organic life, with microbial respiration as a biomarker: simple organic compounds (sodium formate, sodium lactate, glycine, alanine, and calcium glycolate) were added to the Martian regolith. In the presence of forms of life (bacteria, algae…), the organics would have been decomposed, and CO_2 would be released (Levin and Straat 1976a, 293-311). Preliminary sterilization of the Martian regolith at 160°C would have eliminated the response confirming the biological response.

Viking 1, LR test, July 30, 1976, 7h 30 pm, tenth Martian day that the probe was on Mars (Sol 10): a high release of $^{14}CO_2$ (or other carbon-based gas, most likely CO_2) was registered after organic substances were added. Preliminary sterilization at 160°C reduced the response to almost zero (Fig. 1-3).

The experiment was repeated several times (Levin and Straat 1976b, 1322-1329):

Around 50 °C the response was around zero.

A sample under a rock of Martian soil (to prevent the possible "activation" of oxidizing substances by the solar UV) was positive.

A regolith sample left for more than 100 sols inside the probe did not react (the "starvation test").

The same happened in the samples taken 3000 km away (Viking 2). Everything that happened confirmed the biological response (Levin 2007, 39-47; Levin, and Straat 2016, 293-311).

But a few days later, Viking's GCMS (gas chromatograph – mass spectrometer) found no traces of organic compounds in the Martian regolith. At this point many scientists, after the data from GCMS, hypothesized that the release of carbon dioxide in the LR experiments would be the result of the action of strongly oxidized compounds (superoxides and peroxides) that could be present in the Martian regolith. The plaque placed in front of the Viking Lander Exhibit in the Smithsonian National Air & Space Museum, Washington D.C., USA, summarizes very well the state of the art at the end of the 20th century: "*The biological experiments on the Viking Landers did not detect any positive signs of life or any of the organic compounds that are so abundant on Earth. The tests suggested that Martian surface chemistry may in fact result in the destruction of organic material…*"

But it was at the end of the 20th century that a scientific article in the prestigious journal Science reopened the question of life on Mars.

Rocks from Mars rain on the Earth

We have dozens of Martian rocks in their entirety and hundreds of fragments of Martian rocks on our hands, as well as fragments from the Moon. When a large asteroid hits Mars, pieces of the asteroid and Martian soil are catapulted into space. Following chaotic orbits, the fragments enter solar orbit and, passing close to Earth's orbit, can be attracted to our planet and fall as meteors, where we collect them as meteorites (Fig. 1-4).

Fig. 1-4: "Tissint", a Martian rock. On the left, the melting crust produced by crossing the Earth's atmosphere: the rock on its surface reaches more than 1000°C. On the right its inner face (64X), "megacrystals" of ovoidal olivine, characteristic of this Martian rock, immersed in the clear matrix of small crystalline pyroxene. Giorgio Bianciardi, private collection.

In 1996, data from a Martian meteorite, ALH84001, shocked the scientific community interested in the question of life on Mars (McKay, Gibson, Thomas-Keprta, Vali *et al*. 1996, 924-930). ALH84001 is a coarse-grained, cataclastic orthopyroxenite containing coarse-grained inclusions of chromite, carbonates and plagioclase (felspathoid glass and maskelynite) and submicron crystals of iron oxides and sulfides, predominantly magnetite and pyrrhotite with secondary Fe-Mg-Mn-Ca carbonate. They are the oldest of the Martian meteorites we have on our hands. They derive from a basaltic magma that settled on, or near to the Martian surface, crystallizing from molten rock about 4.5 billion years ago. About 15 million years ago a major asteroid impact on Mars threw ALH84001 into space, and about 13,000 years ago it fell onto an ice field in the Allan Hills, Antarctica. The rock from Mars contains a lot of organic compounds, and we are sure that they are of indigenous origin from Mars, not from terrestrial contamination. Interestingly, the presence of carbonate globules reveals that the rock was immersed in liquid water on Mars (at 18°C!, see Halevy, Fischer, and Eiler 2011, 16895–16899).

Numerous biomarkers appeared to be present in the Martian meteorite:

1) Isotopic signature $\delta^{13}C$, a measure of the ratio of stable isotopes $^{13}C/^{12}C$ = 17 – 42 ‰, which was consistent with organic compounds derived from a biological entity (biological processes preferentially take up the lower mass isotope through kinetic fractionation).

2) Presence of magnetite + iron sulfide. Simultaneous presence of oxidized and reduced iron, difficult to obtain inorganically, but easily explained if from biological processing.

3) Presence of 100% pure magnetite, very difficult to explain if of inorganic origin, but easily explainable if from biological processing.

As the years went by, these claims of possible traces of Martian biological activity within the meteorite have been challenged by alternative hypotheses that include a variety of non-biological active agents. Studies of this piece of Martian rock continue, with some confirming the biology and others denying the biological hypothesis (biological origin of magnetite in ALH84001: magnetite crystals in ALH84001 is a truncated hexoctahedron, the same of magnetobacteria on Earth, see Thomas-Kerpta, Clemett, Bazylinsk, Kirschvink *et al.* 2002, 3663-3672; Thomas-Kerpta, Clemett, Wentworth, McKay *et al.* 2009, 1-2; abiotic origin of magnetite in ALH84001, see Treiman, and Essene 2010, 1159-1159; abiotic origin of the organic compounds in ALH84001, see Steele, Benning, Wirth, and Schreiber 2022, 172-177).

The new face of Mars

The interpretation by DS McKay *et al.* remains controversial, however this little piece of Martian rock immediately caused a burst of interest in astrobiology in the scientific world: the first Astrobiology Science Conference, NASA Ames Research Center, USA, held in 2000; the first European Workshop on Exo-/Astrobiology, Frascati, Italy, held in 2001.

These may be considered as constituting the birth of modern Astrobiology.

It was therefore no coincidence that new probes flew to Mars at the beginning of this century. They provided new data on the planet's potential habitability. Following the water was the imperative of these new NASA probes, and a huge amount of water ice was discovered. A massive expanse of water ice was found immediately beneath the surface at the Martian equator (Fig. 1-5); a polar base of water ice, 600 km large, 3 km deep, at the Martian South Pole (Fig. 1-6); craters filled with ice water and fresh snow (Fig. 1-7).

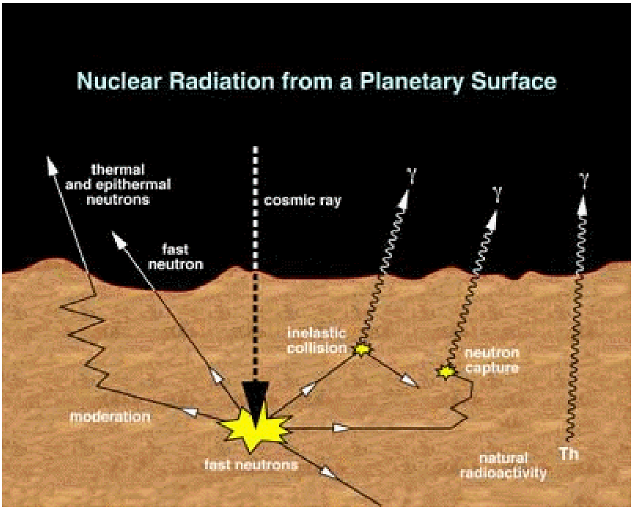

Fig. 1-5: Mars Odyssey, early 2000s. Martian equator, 1 meter below the surface a large amount of water ice. Credit: NASA.

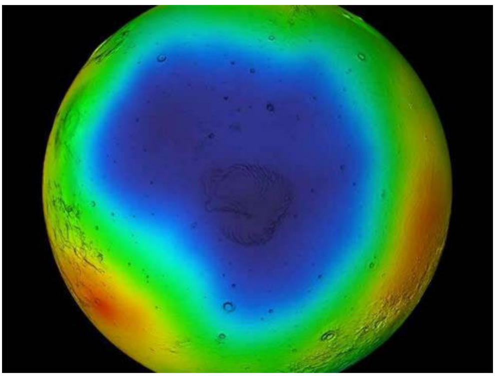

Fig. 1-6: Mars Odyssey, early 2000s. Mars' South Pole- a huge amount of frozen water (immediately underneath a thin layer of solid carbon dioxide). Credit: NASA.

Fig. 1-7: Mars Express, the Korolev crater, Mars, today. Frozen water with fresh snow in a crater: Long ago there was an 80 km water lake (Brothers and Holt 2016, 1443-1449). Credit: ESA.

New aspects of Mars which indicated very favorable conditions for life had been discovered. We may recall the presence of methane in the Martian atmosphere, a possible biomarker and, perhaps, formaldehyde, another possible powerful biological signature, which were discovered by Mars Express, ESA (Formisano, Atreya, Encrenaz, Ignatiev *et al.* 2004, 1758-1761); the discovery of deep lakes of liquid water or melting ice (Lauro, Pettinelli, Caprarelli, Guallini *et al.* 2020, 63-70); a number of organic molecules, up to 10C, discovered on Mars, by NASA Curiosity and Perseverance Rovers (Sharma, Roppel, Murphy, Beegle *et al*. 2023, 724-732) and signs of liquid water on the present-day Mars were identified by the Chinese Martian Rover, Zhurong (Qin, Ren, Wang, Liu *et al.* 2023, eadd 8868).

A return to the Levin's Labeled Release test

At the end of the first decade of the 2000s, many clues were indicating situations that could suggest a Martian environment suitable for hosting life forms. In the meantime, many authors returned to the Viking Labeled Release test, taking LR evidence of life on Mars very seriously.

In comparing the sensitivity of the Gas Chromatography-Mass Spectrometer (GCMS) aboard the Vikings and the LR test, Viking's GCMS turned out to be unable to recognize pyrolysis products of <1 million bacteria / g, while LR proved capable of recognizing 10 bacteria / g). Consequently, it had become apparent that GCMS on Mars had a huge sensitivity problem (Glavin, Schubert, Botta, Kminek *et al.* 2001, 1-5).

The Phoenix lander (NASA) descended on Mars on May 25, 2008 discovering the presence of perchlorate on Mars. Repeating the GCMS analysis in the presence of perchlorate in a location that can recall Mars (Atacama Desert, Chile), the GCMS denied the presence of organics, except some chlorinated derivatives of methane, even if bacteria and fungi were present. In effect, the GCMS in the presence of perchlorate destroys organic compounds, producing chlorinated derivatives of methane. On Mars, indeed, the GCMS recognized the "strange" presence of chlorinated derivatives: the organics destroyed by the GCMS itself. The GCMS actually recognized the presence of organic compounds on Mars (Navarro-Gonzalez, Vargas, de La Rosa, Raga *et al.* 2010, 1-11)!

The Viking LR showed a highly periodic gas release whose signals indicated a rhythm: 24.66 h, the length of a Martian day (sol). Was this possibly a circadian rhythm? In the case that it was, there would be a clear biomarker. But the temperature inside the Lander also showed a 2°C temperature fluctuation (a reflection of the temperature variation during the day on Mars).

If the circadian rhythm of the released gas was determined by temperature, it would be a chemical phenomenon and not a biological one: "lacking the smoking gun", wrote the authors (Miller, Levin and Straat 2002, 96-107).

To address this, we applied a chaotic analysis (Appendix 1.1) of CO_2 fluctuations, at first in a small group of data (Bianciardi 2004, 105-105), and then in all LR data: seven LR tests from 16,000 test points (on Mars), compared to fluctuations in biological (from Earth) and abiotic data (Bianciardi, Miller, Straat and Levin 2012a, 14-26): a cluster analysis showed that the active LR data on Mars overlapped perfectly with the biological data and were clearly separated from all abiotic data ($p < 0.001$), thus confirming the evidence that LR revealed the presence of living beings on Mars (Table 1-1).

Interestingly, by focusing on the first six days (sols) on Mars and performing a phase space diagram with nonlinear indices, and comparing the temperature values inside the Viking probe with the carbon dioxide fluctuation inside the Viking during an active LR test—as well the fluctuation of carbon dioxide released by bacteria on Earth in a LR test simulation—, we can observe that the temperature and CO_2 data differed profoundly, while the LR trajectories and biological ones overlap perfectly. The "smoking gun" sought out by Miller et al. 2002 was found: the temperature

Cluster 1: Controls

Case	Distance	Variables	Minimum	Mean	Maximum	SE
VL2C4	0.464	LZ	0.294	0.790	1.379	0.115
VL1C2	0.593	H	-0.984	-0.706	0.111	0.144
VL1C4	0.479	λ	0.000	0.724	2.404	0.276
VL2C5	0.966	K	-1.434	0.534	1.449	0.325
BIOL 6	0.311	BDS	-2.010	-0.721	0.190	0.293
DT VL2C3	0.790	τ	-0.645	-0.508	-0.156	0.076
VL1 Atmo. temp	0.413					
Pre-inj radioactivity	0.494					

Cluster 2: Active LR (Mars) and Biological (bacteria and rats) tests

Case	Distance	Variables	Minimum	Mean	Maximum	SE
BIOL5	0.534	LZ	-2.136	0.902	-0.080	0.248
VL1C1	0.285	H	-0.218	0.806	2.190	0.320
VL1C3	0.409	λ	-1.202	0.828	-0.219	0.133
VL2C1	0.544	K	-1.656	0.610	0.673	0.277
VL2C3	0.622	BDS	0.664	0.824	1.291	0.084
VL2C2	0.587	τ	-0.174	0.581	3.304	0.470
Rat temp	1.354					

Variable	Between SS	df	Within SS	df	F-ratio	p<
LZ	10.689	1	3.311	13	41.975	.001
H	8.534	1	5.466	13	20.299	.001
λ	8.988	1	5.012	13	23.312	.001
K	4.889	1	9.111	13	6.976	.05
BDS	8.908	1	5.092	13	22.745	.001
τ	4.427	1	9.573	13	6.011	.05
** TOTAL **	46.436	6	37.564	78		

Table 1-1. K-means Cluster Analysis of averages across sols of all detrended data sets for all cases. Complexity variables discriminated two clusters (first and second panels): cluster members (individual experiments and data series) were shown with distances from the centroid for each experiment, as well as means, SEs (Standard Errors), and ranges for each discriminating complexity variable. Bottom: F-ratios and p values for each discriminating complexity variable.
VL = Viking Lander data. Biol 5 (bacteria, active), Biol 6 (sterilized).
It may be seen that the various experiments sort into what can be labeled as controls or physical data (Cluster 1) and as active LR (Mars) or biological data (Cluster 2). Discriminant analysis indicated that the two clusters differed significantly on the complexity variables ($p<.001$), supporting that LR revealed the presence of living beings on Mars.

did not drive the CO_2 fluctuations during LR tests and, furthermore, the identity between the LR data obtained on Mars and the same on Earth with bacteria was surprising (Fig. 1-8, Bianciardi, Miller, Straat and Levin 2012b, 501-502).

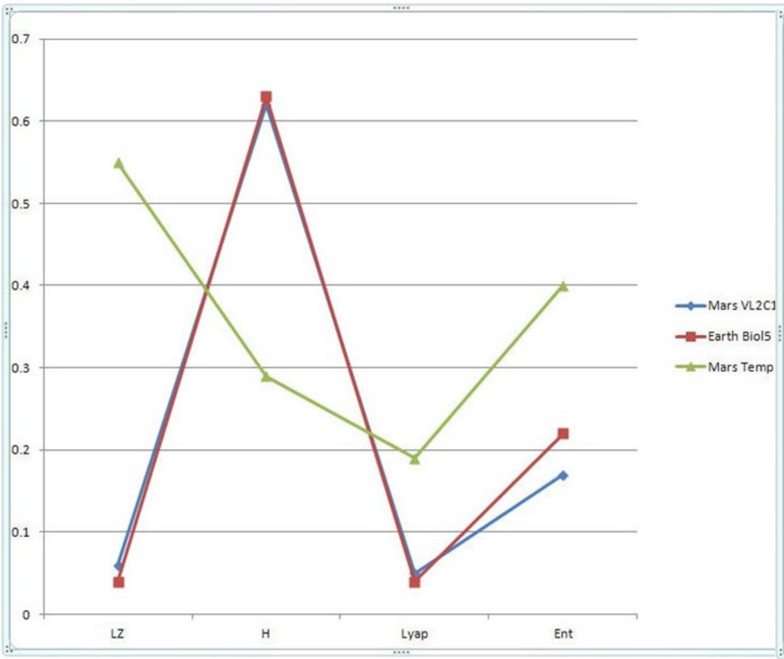

Fig. 1-8: Phase space of the chaotic indices, LR active test performed on Mars or on Earth (bacteria), temperature inside the Viking probe. A terrestrial biological sample in the LR (Biol 5 test: bacteria) has a behavior of the chaotic indices perfectly superimpose on what was observed on Mars during the first 6 days (sols) on Mars. Note also that the trajectory of the temperature vastly differed from the one of the LR tests performed on Mars: the rhythm of the CO_2 oscillation observed by Miller in 2002 was a real circadian rhythm, it was not due to the variation of temperature. For the meanings of the chaotic indices, see Appendix 1-1.

Stromatolites on Mars?

During the first decade of the 2000s, the Martian rovers, 'Opportunity' and 'Spirit' (NASA), wandered the sands of the Red Planet.

The debate began early on. Squyres (Squyres, Grotzinger, Arvidson, Raymond *et al.* 2004, 1709-1714), when describing images from the NASA Rover, 'Opportunity', reported clear evidence of an ancient aqueous environment on Meridiani Planum and the presence of hematite-rich spherules, that were named “blueberries”. According to the authors these were concretionary in nature, and abiotic structures. However, contrastingly, Parro (Parro, Rodriguez-Manfredi, Briones, Compostizo *et al.* 2005, 729-737) pointed out that in extreme acidic environments, there emerged morphological evidence of the Martian ‘blueberries’ as related to communities of chemolithoautotrophic bacteria, just like the ones still living on Earth (Weber, Weber, Spanbauer, Wacey *et al*. 2012, 747-750). Such were the conditions the Meridiani Planum in the early Mars; on Earth they are controlled by iron biogeochemistry, that is, ferric-iron-enriched sediments resulting in goethite, hematite and jarosite—the same minerals analogous to those found in Meridiani Planum, said the authors, and, also, by Rizzo and Cantasano (Rizzo and Cantasano 2009, 267-280).

We may recall that microbialites, including stromatolites (Fig. 1-9), are an often cited target for the search for life on Mars (McKay 2004, 283-288; Jepsen, Priscu, Grimm, and Bullok 2007, 342-354; Clarke, and Stocker 2013, 413-423). They are rocks built by unicellular life forms and constitute the oldest evidence of life forms, starting 3.7 billion years ago on Earth (Nutman, Bennett, Friend, Van Kranendonk *et al*., 2016, 535-538): an anoxic Earth, like Mars today. From a geological point of view, a microbialite is a benthic sedimentary deposit made up, for example, of carbonate mud formed with the mediation of microbes. From the biologists’s

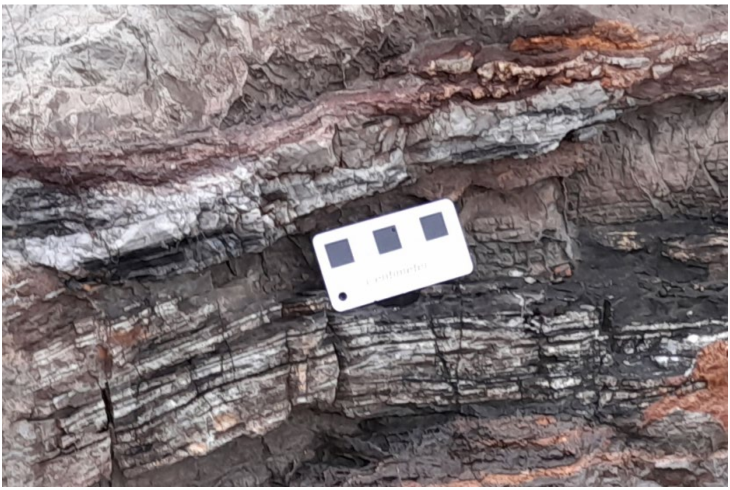

Fig. 1-9: Below the marker- a typical aspect of a stromatolite (dating back to 1.5 billion years ago). Location: Taihangshan, China.

point of view, a microbialite, including a stromatolite, is a sort of "exoskeleton" built by cyanobacteria. The meaning, however, is the same: life.

In effect, several authors for many years have been suggesting evidence of microbialites in the Martian outcrops, always starting from the images shot by the Opportunity and Spirit Rovers.

In 2009, in a study of the Martian outcrops at sub-millimeter (0.1-1 mm) to centimeter scale, at the Opportunity site, Rizzo reported observations of segmented, lamina-bounded, sedimentary structures at the Meridiani Planum, that, along with other findings, could be interpreted as evidence of stromatolites on Mars (Rizzo, and Cantasano 2009, 267-280). These scholars collected a set of enlarged Martian microscopic imagery (MI) samples, showing a textural similarity to several samples of stromatolites (see Fig. 2-19 to 2-24).

In 2014 and 2015, fractal morphometric analyses were performed using a computer, with an automatic detection of contours of 25,000 Martian microstructures compared with 15,000 terrestrial microbialite/stromatolite microstructures. The analysis revealed evidence of stromatolites in the Martian outcrops at the Opportunity site (Bianciardi, Rizzo, and Cantasano 2014, 419-433), as well as at the Spirit site (Bianciardi, Rizzo, Farias, Cantasano 2015, 1-8) and were subsequently confirmed further (see Chapter 3).

In 2015, studying the Martian outcrops on a centimeter to meter scale, on the Curiosity Martian Rover site, N. Noffke described the presence of "erosional remnants and pockets," "mat chips," "roll-ups," "desiccation cracks," and "gas domes" which do not have a random distribution but were arranged in spatial associations and temporal successions similar to the "growth of a microbially dominated ecosystem(among which stromatolites) that thrived in pools that later dried completely" (Noffke 2015, 1-24).

In 2016, Ruff and Farmer, when studying the outcrops and regolith at the Spirit site, identified opaline silica in an ancient volcanic hydrothermal setting in the Gusev crater, which strongly resembled the environment of active hot spring/geyser discharge channels, including complex sedimentary structures produced by a combination of biotic and abiotic processes (Ruff and Farmer 2016, 1-10).

More evolved life beings on Mars?

Over the last years, data showing evidence of more evolved life on Mars having been present in the past or, perhaps, also today, is increasing.

In 2016, over 2000 experts in the faculties from nearly 1,000 universities were invited to observe images shot by the Martian Rovers, and of fungi or lichens from Earth. Forty Biologist experts in fungi and lichens, and thirty Geologist experts in mineralogy and/or geomorphology participated, observing 25 photos from Mars and 5 from Earth. The online rating system allowed each participant to rate each photo on a 1 to 4 scale, on the probability of life: 1 (No/0%) - 2 (33% Probability) - 3 (66% Probability) - 4 (Yes/100%). Statistical tests demonstrated that the majority of the experts, in both fields, assigned a probability of fungi or lichens on Mars equal to 66% or 100% (Joseph 2016, 1-25).

In 2017, at the Lunar and Planetary Society meeting, Krupa described images from the Spirit Rover's Pan Cam drawing attention to the presence of flowing water with a "thin layer of green material" and "green spherules" that resembled photosynthetic algae in the Martian regolith (Krupa 2017, 2711-2711).

In 2019, Trainer *et al*., reported Martian atmospheric oxygen to be continually replenished despite its five year half-life, despite leakage into space, and its increased levels in spring and summer. The presence of photosynthesizing lichens and algae should be able to explain it better than other hypotheses (Trainer, Wong, McConnochie, Franz *et al*. 2019, 3000-3024).

In 2020 and 2021, Rizzo described the presence not only of stromatolite-like structures on the Martian outcrops, but, through observing details taken down with the Mars Hand Lens Imager (MAHLI) camera on board Curiosity, the presence of elongated, white curved spots- lozenge-shaped- ending on both sides in a point ("rice grains"). These were reminiscent of Cyanophyta or of certain green algae such as Gymnocodiaceae or some forms of Euglenaceae (Rizzo 2020, 1-12; Rizzo, Armstrong, Hua, Cantasano *et al*. 2021, 97-128). Interestingly, these microstructures were observed in Aeolis Palus on Gale Crater, which houses geological remains of an ancient freshwater lake. The same microstructures were previously identified as mineral crystals, like gypsum (Kah, Stack, Eigenbrode, Yingst *et al.* 2018, 431-439). To distinguish between the hypothesis, Bianciardi (Bianciardi, Nicolò, Bianciardi 2021, 70-79), using fractal analysis, showed that the Fractal Dimension, D0, and Entropy, D1, of the Martian lozenge microstructures differ from that of gypsum (negative control) with high statistical significance ($p < 0.01$; $p < 0.01$). By contrast, the fractal dimension of the Martian lozenge microstructures overlapped with that of the extremophile unicellular alga *Euglena mutabilis*, (see Chapter 3).

In 2021, Elewa described the possible presence of fossilized formations similar to tube worms (Elewa 2021, 29-37).

In 2021 Armstrong, applying morphometric approaches based on principal component analysis, reported the presence of stalked spheroids disseminated in the Martian outcrops on images from the Opportunity rover. He claimed they can be better explained as the reproductive podetia of a lichen (Armstrong 2021, 15-21). In 2022, Armstrong also described forms resembling sponges or corals in Gale Crater, on Mars (Armstrong 2022, 4-12).

In 2023, other in-depth analyses of the images of Martian outcrops were conducted by us (Rizzo, Bianciardi, Armstrong), which are the theme of the following chapters of this book.

It is clear that the data which have been collected concerning the possible presence of living beings on Mars in the past or even in the present, are now so numerous that they cannot be denied.

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